

First Pakistani report of *Erysiphe betae* on the invasive weed *Chenopodium ambrosioides*

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ABSTRACT—During September–October 2017, powdery mildew symptoms were observed on both surfaces of leaves of *Chenopodium ambrosioides* in Abbottabad, Malakand, and Upper Dir districts of Pakistan. The causal agent was identified as *Erysiphe betae*, based on its asexual morphology and chasmothecia, and its identity was confirmed by molecular data. This is the first Pakistani report of *Erysiphe betae* on this host.

KEY WORDS—*Amaranthaceae*, *Erysiphaceae*, mexican tea, new record

Introduction

Fungi belonging to *Erysiphales* are widely distributed all over the world and cause serious economic damage on wild and cultivated plants. This order comprises the single family *Erysiphaceae* (powdery mildews) represented by 17 genera and c. 820 species (Braun & Cook 2012). Powdery mildews infect 9839 angiosperm species in 1617 genera, 196 families, and 44 orders (Amano 1992, Takamatsu 2004), including numerous economically important plants. Various powdery mildews may cause serious economic damage on wild and cultivated plants in Pakistan (Burni & al. 2010, Mukhtar & al. 2012,

2013). Nineteen of the 56 powdery mildew species reported from Pakistan represent the most common genus, *Erysiphe*, which infects 52 hosts in 19 plant families (Ahmad & al. 1997, Burni & al. 2010, Mukhtar & al. 2013). This includes *Erysiphe betae* on *Chenopodium botrys* L. and *Spinacia oleracea* L. (*Amaranthaceae*; Amano 1986).

In 2017, a powdery mildew appeared on the leaves of *Chenopodium ambrosioides* in the districts Abbottabad, Malakand, and Upper Dir, Pakistan. The infected leaf surfaces were fully covered with white powdery mycelial masses, asexual conidia, and sexual chasmothecia indicating progressed stages of infection and severity.

Materials & methods

Sample collection

During phytopathological surveys during September–October 2017, we observed powdery mildew infections on leaves and stems of *Chenopodium ambrosioides* in the districts Abbottabad, Malakand, and Upper Dir, Khyber Pakhtunkhwa, Pakistan. The infected plants were shade dried on blotting paper and protected in brown envelopes for future use. The specimens are deposited at the herbarium of the University of Peshawar, Khyber Pakhtunkhwa, Pakistan (PUP) and Department of Botany, University of the Punjab, Lahore, Punjab, Pakistan (LAH).

Morphology

We first examined infected leaves under a Labomed CSM2 stereomicroscope, and then prepared using lactic acid. We examined the hyphae on the host; hyphal appressoria; conidial, conidiophore, and chasmothecial shapes and sizes; and ascospores and asci under a Nikon YS 100 microscope. Measurements (20 repetitions per structure) were recorded using a Lomo Filar Eyepiece Micrometer AM9-2-15x on a Zeiss microscope. Micrographs were made using a HDCE-5X digital camera.

DNA extraction, PCR amplification, phylogenetic analysis

Dried powdery mildew infections were scraped off from fresh fungal specimens with sterile razor blades, ground in liquid nitrogen, and stored in Eppendorf tubes at -18°C . DNA was extracted using GeneJET Plant Genomic DNA Purification Mini Kit #K0791 according to the manufacturer's instructions. The Internal Transcribed Spacer (ITS) region was amplified using PMITS1/PMITS2 primers (Cunnington & al. 2003) and then commercially sequenced by Tsingke in China. Raw sequence data were edited using BioEdit (Hall 1999). The ITS sequences were BLAST searched against the GenBank database (www.ncbi.nlm.nih.gov) and aligned using Muscle E multiple alignment tool within MEGA v. 7.0 (Kumar & al. 2016). Twenty ITS sequences were analyzed using the maximum likelihood (ML) method based on the Tamura 3-parameter model (Tamura 1992). The initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise

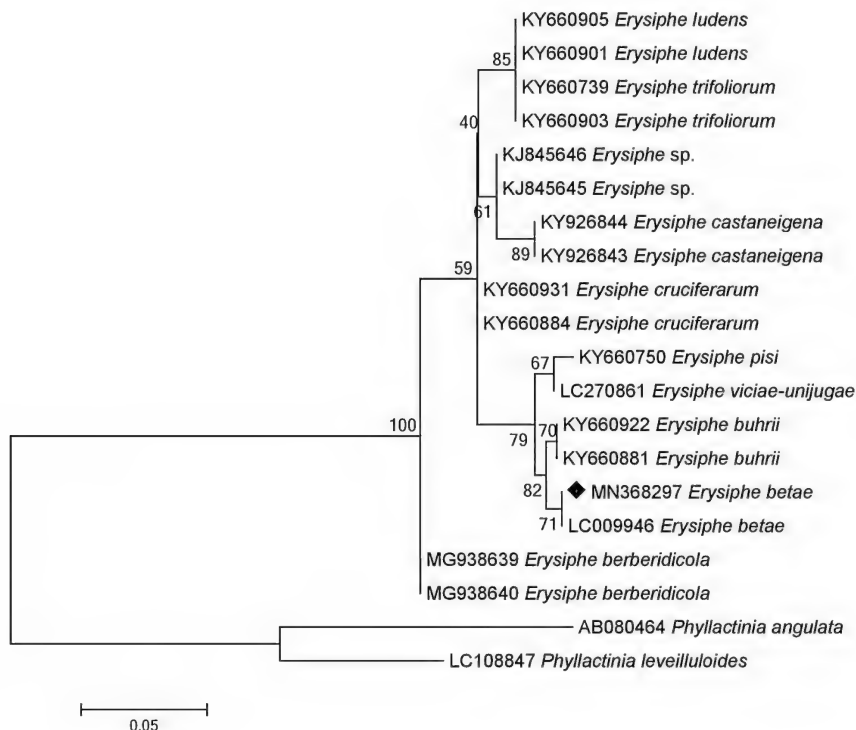


FIG. 1. Phylogenetic analysis of the ITS region for 18 *Erysiphe* sequences, with *Phyllactinia angulata* and *P. leveilluloides* as outgroup. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. The tree with the highest log likelihood (−470.6292) is shown, and the amplified sequence from Pakistan is denoted by ◆.

distances estimated using the Maximum Composite Likelihood (MCL) approach. Evolutionary analyses were conducted in MEGA6 (Tamura & al. 2013). in the phylogenetic analyses. After elimination of gaps and missing data, the final dataset comprised 134 positions, and *Phyllactinia angulata* (E.S. Salmon) S. Blumer and *P. leveilluloides* Moreno-Rico & U. Braun served as outgroup. All sequences were aligned and trimmed at conserved sites from both 5' and 3' ends. The phylogenetic tree with the highest log likelihood (−470.6292) is shown in FIG. 1; the percentage of trees in which the associated taxa clustered together is shown next to the branches, and the tree is drawn to scale, with branch lengths measured in the number of substitutions per site.



FIG. 2. *Erysiphe betae* (PUP Bot.01). A. Infected plant, *Chenopodium ambrosioides*; B. Fungal mycelium under stereomicroscope; C. Conidia; D. Conidiophore; E. Chasmothecium; F. Germinating Conidium; G. Asci with ascospores; H. Ascospores; I. Chasmothecial appendage. Scale bars: A = 1 cm, B = 2 mm, C, D, F, G = 15 μ m, E, H, I = 10 μ m.

Taxonomy

Erysiphe betae (Vaňha) Weltzien, Phytopathol. Z. 47: 127 (1963)

FIG. 2

Mycelium amphigenous, forming dense, thick, white patches; hyphae hyaline, smooth, thin-walled; hyphal appressoria nipple-shaped to somewhat lobed, 2–5 μm diam. Conidiophores arising from the top of mother cells, $43\text{--}87 \times 3.5\text{--}7.5 \mu\text{m}$; foot cells cylindrical, straight or sometime curved, $22\text{--}61 \times 4.5\text{--}8 \mu\text{m}$, constricted at the basal septum, followed by 1–2 shorter cells; conidia formed singly, ellipsoid-ovoid to cylindrical-doliiform, $14.5\text{--}51 \times 7\text{--}12 \mu\text{m}$; germ tubes arising from one end, tips slightly swollen; Chasmothecia scattered to gregarious, globose to subglobose, light to dark brown, $48.5\text{--}104 \mu\text{m}$ diam.; peridium cells small, $12\text{--}27 \mu\text{m}$ diam., irregularly polygonal; appendages numerous, mycelioid, irregularly branched, septate, thin-walled, at first hyaline but pigmented when chasmothecia mature, $29\text{--}119 \times 2.5\text{--}4.5 \mu\text{m}$; asci 3–5, ellipsoid-obovoid, $28\text{--}49.5 \times 19.5\text{--}29.5 \mu\text{m}$, stalked, 4–5-spored; ascospores globose-ellipsoid to ovoid, $8.5\text{--}18.5 \times 5.5\text{--}10.5 \mu\text{m}$, colorless.

SPECIMENS EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA, Malakand Division, Malakand district, 844 m a.s.l., on *Chenopodium ambrosioides* L. (Amaranthaceae), 19 September 2017, asexual morph, A. Anwar AA#4 (PUP Bot.01; GenBank MN368297); Upper Dir district, 844 m a.s.l., on *C. ambrosioides*, 29 October 2017, asexual and sexual morphs, A. Anwar AA#5 (PUP Bot.02); Hazara Division, Abbottabad district, Ayubia National Park, 2438 m a.s.l., on *C. ambrosioides*, 30 October 2017, asexual morph, N.S. Afshan NSA#34 (LAH 35658).

Phylogenetic results

The NCBI BLASTn analysis showed that our ITS sequence (MN368297 *Erysiphe betae*) closely matched (99.54% identity) a sequence of *E. betae* (LC009946) on *Beta vulgaris* from Japan (Takamatsu & al. 2015). The 20 nucleotide sequences used in the phylogenetic analysis included *Phyllactinia angulata* (AB080464) and *P. leveilluloides* (LC108847) as outgroup. The final aligned data set contained 818 positions of which 567 were conserved, 202 variable and parsimony uninformative, and 49 parsimony informative sites. Maximum likelihood (ML) analysis clustered the Pakistan *E. betae* collection (MN368297) with the Japanese *E. betae* collection (LC009946) with a 71 bootstrap value. Based on these results, we assigned the name *Erysiphe betae* to the powdery mildew pathogen on *Chenopodium ambrosioides* in Pakistan.

Discussion

Worldwide, three *Erysiphe* spp. (including *E. betae*), two *Leveillula* spp., and three anamorphic powdery mildew species have been reported on *Chenopodium ambrosioides* (Fungus-Host Database <https://nt.ars-grin.gov>, 29 May, 2018, Braun & Cook 2012). From Pakistan, only “*Erysiphe communis* (Wallr.) Schltdl.” (nom. rej.) on *Chenopodium botrys* and *Spinacia oleracea* and *Leveillula cylindrospora* U. Braun and *L. taurica* (Lév.) G. Arnaud on *Chenopodium murale* L. have been reported. *Chenopodium ambrosioides* is reported here as a new host record for *Erysiphe betae* in Pakistan.

Previously, only asexual morphs of *Erysiphe* have been reported on *Chenopodium* species from Asia and Europe, whereas sexual fruiting bodies (chasmothecia) have been reported only from India and Japan (Braun & Cook 2012). We now report both asexual and sexual morphs of *Erysiphe betae* from Pakistan. We found only the asexual morph in the Abbottabad and Malakand districts, but additionally collected the chasmothecia in the Upper Dir district. Previous reports from Pakistan of *Erysiphe* on *Chenopodium botrys* (as “*E. communis*,” Amano 1986) is probably allocable to *E. betae*. Francis & al. (2007), who analysed ITS rDNA sequences retrieved from European (UK) and North American (USA) collections of *E. betae* on sugar beet, confirmed the involvement of a single recognizable *Erysiphe* species more closely allied to *Erysiphe heraclei* DC. than to *E. polygoni* DC. Takamatsu & al. (2015) included in their phylogenetic analyses a sequence obtained from *E. betae* on *Beta vulgaris* L. with corresponding results, but sequences retrieved from *E. betae* on *Chenopodium ambrosioides* are not yet available and still in need of molecular confirmation. Our research represents the first phylogenetic analysis of *E. betae* on *Chenopodium ambrosioides*, which clusters our ITS sequence in a strongly supported clade with a sequence of *E. betae* on *Beta vulgaris* (FIG. 1).

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Literature cited

- Ahmad S, Iqbal SH, Khalid AN. 1997. Fungi of Pakistan. Sultan Ahmad Mycological Society of Pakistan, Lahore, PK.
- Amano K. 1986. Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo.

- Amano K. 1992. Notes on the host range and geographical distribution of *Podosphaera*. Transactions of the Mycological Society of Japan 33: 139–148.
- Braun U, Cook RTA. 2012. Taxonomic manual of the *Erysiphales* (powdery mildew). CBS Biodiversity Series 11. 707 p.
- Burni T, Jamil F, Jabeen M. 2010. *Erysiphales* of Peshawar valley, Khyber Pakhtunkhwa, Pakistan. Pakistan Journal of Plant Sciences 16(1): 11–14.
- Cunnington JH, Takamatsu S, Lawrie AC, Pascoe IG. 2003. Molecular identification of anamorphic powdery mildews (*Erysiphales*). Australasian Plant Pathology 32: 421–428. <https://doi.org/10.1071/AP03045>
- Francis SA, Roden BC, Adams MJ, Weiland J, Asher MJ. 2007. Comparison of ITS sequences from UK and North American sugar-beet powdery mildews and the designation of *Erysiphe betae*. Mycological Research 111(2): 204–212. <https://doi.org/10.1016/j.mycres.2006.10.010>
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. <https://doi.org/10.1017/S0953756203008517>
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874 <https://doi.org/10.1093/molbev/msw054>.
- Mukhtar I, Khurram R, Hannan A, Hayat Z. 2012. First report of powdery mildew of *Cucurbita moschata* Duch. caused by *Golovinomyces cichoracearum* DC. in Neelum Valley, Azad Kashmir. Plant Disease 96(6): 906. <https://doi.org/10.1094/PDIS-08-11-0642-PDN>
- Mukhtar I, Mushtaq S, Khokhar I. 2013. First report of powdery mildew on *Dahlia* (*Dahlia variabilis*) caused by *Golovinomyces cichoracearum* in Lahore, Pakistan. Australasian Plant Disease Notes 8(1): 1–3. <https://doi.org/10.1007/s13314-011-0025-7>
- Takamatsu S. 2004. Phylogeny and evolution of the powdery mildew fungi (*Erysiphales*, *Ascomycota*) inferred from nuclear ribosomal DNA sequences. Mycoscience 45: 147–157. <https://doi.org/10.1007/s10267-003-0159-3>
- Takamatsu S, Ito H, Shiroya Y, Kiss L, Heluta V. 2015. First comprehensive phylogenetic analysis of the genus *Erysiphe* (*Erysiphales*, *Erysiphaceae*) I. The *Microsphaera* lineage. Mycologia 107: 475–489. <https://doi.org/10.3852/15-007>
- Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and Evolution 9: 678–687. <https://doi.org/10.1093/oxfordjournals.molbev.a040752>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. <https://doi.org/10.1093/molbev/mst197>